

continuation application reflect all amendments entered by the Examiner in the parent application.

In compliance with 35 U.S.C. §120, the amendment to the specification adds specific reference to the priority application. The amendment adds no new matter.

In response to the Advisory Action mailed in the parent application on October 17, 2001, Applicants request that the Examiner consider the following remarks.

All pending claims of the parent application (1-24) were rejected as obvious over a combination of the Heyman et al., Shuman and Pan et al. references cited previously.

Applicants note that the Examiner stated in the first paragraph of the Advisory Action that "Applicants contend that the claimed invention is distinct over previously cited references which delineate and exploit for ligation and/or cloning purposes the catalytic mechanism of topoisomerase because the instant invention comprises a further ligation event to form a covalently joined circular construct." Applicants submit that Applicants' statement in the previous response to which the Examiner refers is "The topoisomerase-mediated covalent joining of the claimed methods results in a linear molecule which requires another ligation event to form a covalently joined circular construct." Applicants wish to clarify for the record that while the statement in the previous response is true, the claims do not, except where it is specifically recited, require such further ligation event.

In the Advisory Action, the Examiner states that "the attachment of topoisomerase to one end or to both ends of a DNA molecule depends upon the presence and accessibility of topoisomerase recognition site(s) on that DNA," and that it therefore would be "a routine matter in the art to insert one or two recognition sites within the DNA in order to provide for a single or double attachment of topoisomerase." Applicants respectfully disagree.

Applicants submit that even if it would be "routine" to perform the individual steps in a given set of manipulations, the performance of those steps is not obvious absent a motivation and the recognition that those steps will achieve a desired goal. Applicants submit that while Shuman, Heyman et al. or Pan et al. may provide motivation to attach topoisomerase to the ends of a molecule to be ligated, they provide no motivation to attach topoisomerase to one end only

of each of a pair of flanking molecules, as required by claim 1, or to attach a topoisomerase to one end only of one flanking molecule, as required by claims 3, 6 and 18. While, it might involve “routine” methods to manipulate the number of topoisomerase molecules on a given nucleic acid, absent a motivation provided by the references themselves there is simply no point in doing so. The fact remains that none of the cited references teaches or suggests any advantage of attaching topoisomerase to one end only of a molecule to be ligated. The mere fact that it can be readily achieved does not make a method involving such attachment obvious. To say otherwise would be to say that because of the routine nature of the required steps, an invention such as the polymerase chain reaction was obvious, which is clearly not the case.

Applicants previously pointed out that none of the cited references provides a directional cloning aspect in a method that involves attachment of topoisomerase to one end only of a flanking molecule. The Advisory Action states that contrary to this assertion, “Heyman et al. describes the 5'-hydroxyl group of an acceptor piece of DNA (either as a fragment or a vector) as a prerequisite for its ability to function as a topoisomerase substrate for ligation” and that “the requisite 5'-hydroxyl group as a substrate for topoisomerase implies a directional control in the topoisomerase ligation reactions.” Applicants submit that the requisite 5'-hydroxyl group does not imply a directionality. A molecule that has a 5'-hydroxyl group on both ends will be ligated in either orientation by the topoisomerase-mediated cloning described by Shuman and by Heyman et al. The directionality imparted by the claimed methods (e.g., claims 2, 3, 6, 13, 19 and their dependents) is provided by the requirement of a flanking molecule with one end only covalently linked to a topoisomerase, and by various combinations of cloning sites or phosphates on the remaining ends of flanking molecules and/or inserts. Applicants therefore submit that neither Heyman et al. nor Shuman nor Pan et al. individually or in combination, teaches or suggests a directional cloning method as recited in claims 2, 3, 6, 13 and 19.

The Advisory Action states that Applicants argue that a transformation step is not explicitly described in the prior art, and then points out that Heyman discloses Western blot analysis of expressed ligation products and that both Shuman and Heyman et al. disclose the use of topoisomerase for ligation reactions for molecular cloning purposes (which implies a transformation step). Applicants wish to clarify that they were not arguing that Shuman and Heyman do not teach a transformation step, but rather that none of the cited references teaches or


suggests the step of transforming a cell with a ligated molecule comprising an insert positioned between a first and a second flanking molecule as recited by claim 1. The constructs transformed in Shuman and Heyman et al. consisted of an insert ligated to a single vector molecule, rather than to a first and a second flanking molecule. Therefore, the cited references do not teach or suggest transforming transforming a cell with a ligated molecule comprising an insert positioned between a first and a second flanking molecule as recited by claim 1.

Finally, the Advisory Action states that "Applicants have provided no data (i.e., regarding the successful generation, characterization, and subsequent expression of any clones in any prescribed or predicted orientations) using such prescribed methods as claimed that would distinguish it from the techniques successfully employed previously in the art, especially those methods which were disclosed and evaluated by Heyman." Applicants intend to address this in the near future with a Declaration under 37 C.F.R. §1.132.

Applicants respectfully request that the Examiner consider the above remarks in examining the application.

Respectfully submitted,

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